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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C12N 15/12, C07K 14/475, C12N 15/11, 15/62, C07K 16/22, C12Q 1/68, G01N 33/53		A1	(11) International Publication Number: WO 99/43811 (43) International Publication Date: 2 September 1999 (02.09.99)
(21) International Application Number: PCT/US99/04142 (22) International Filing Date: 25 February 1999 (25.02.99) (30) Priority Data: 60/075,922 25 February 1998 (25.02.98) US 60/079,567 27 March 1998 (27.03.98) US (71) Applicant (for all designated States except US): THE GOVERNMENT OF THE UNITED STATES OF AMERICA, represented by THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES [US/US]; Suite 325, 6011 Executive Boulevard, Rockville, MD 20852 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): THORGEIRSSON, Snorri, S. [US/US]; 9305 Kingsley Avenue, Bethesda, MD 20814 (US). WOITACH, Joseph, T. [US/US]; 11613 Ashley Drive, North Bethesda, MD 20852 (US). ZHANG, Minghuang [CN/US]; 117 Bates Avenue, Gaithersburg, MD 20877 (US). (74) Agent: WOOD, William, J.; Merchant, Gould, Smith, Edell, Welter & Schmidt, P.A., 3100 Norwest Center, 90 South Seventh Street, Minneapolis, MN 55402-4131 (US).			(81) Designated States: AL, AM, AT, AT (Utility model), AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ (Utility model), DE, DE (Utility model), DK, DK (Utility model), EE, EE (Utility model), ES, FI, FI (Utility model), GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (Utility model), SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: cDNA ENCODING A GENE BOG (B5T OVER-EXPRESSED GENE) AND ITS PROTEIN PRODUCT			
(57) Abstract Nucleic acids that encode novel polypeptides, designated in the present application as "BOG" (<u>B</u> 5T <u>O</u> ver-expressed <u>G</u> ene) are provided. BOG binds to pRb and is over-expressed in a number transformed rat liver epithelial (RLE) cell lines resistant to the growth inhibitory effect of TGF- β 1 as well as in primary liver tumors. Compositions including BOG chimeras, nucleic acids encoding BOG and antibodies to BOG are also provided. Methods of using BOG to modulate pRb-protein interactions and to alter cellular phenotype are further provided.			

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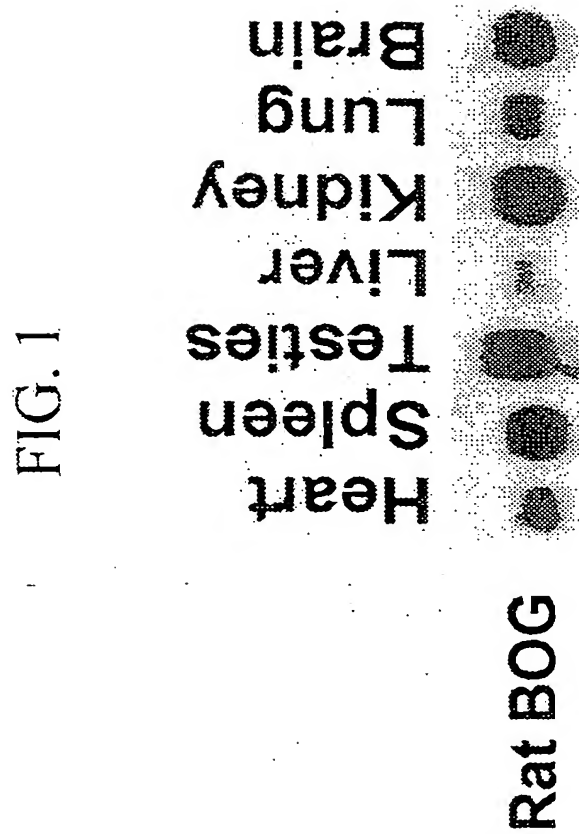


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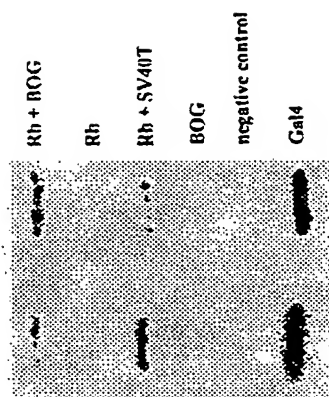


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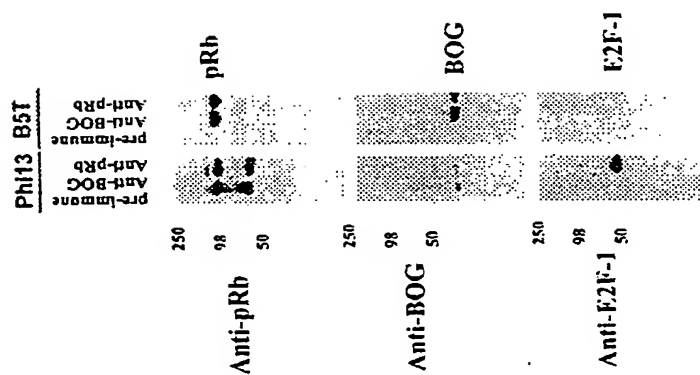


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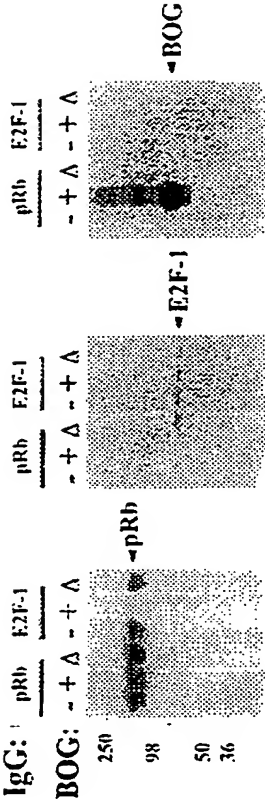
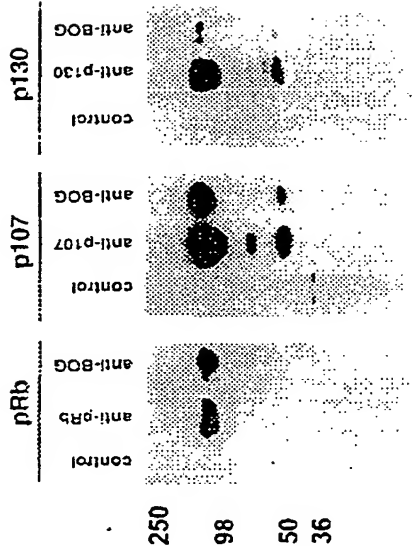


FIG. 2(D) D



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FIG. 3(A)

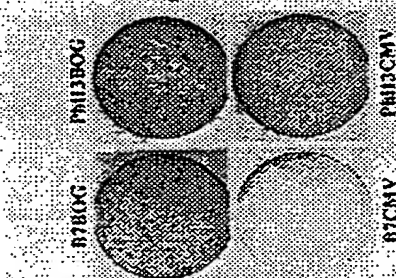


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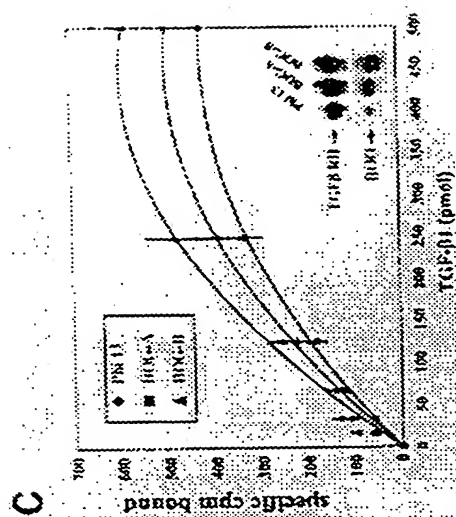


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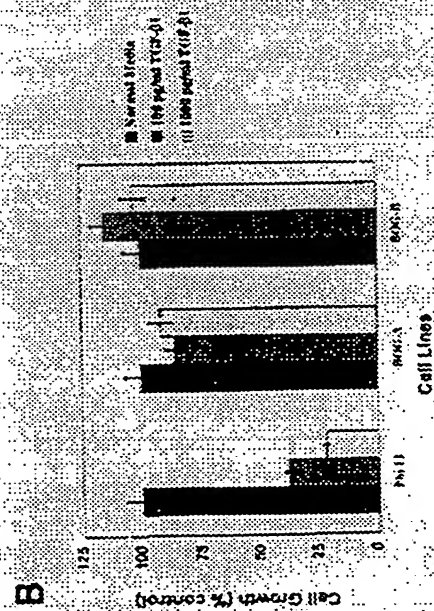
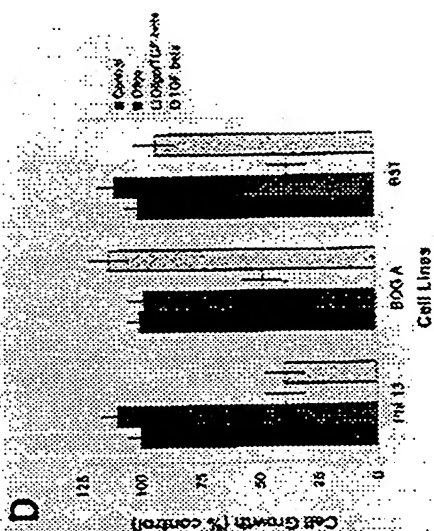
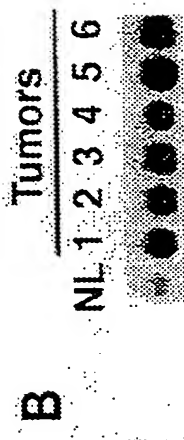
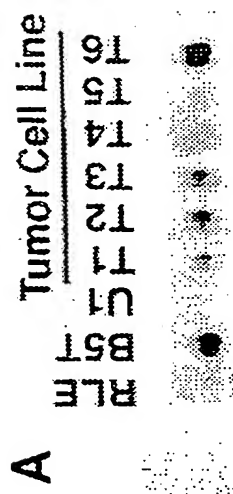


FIG. 3(D)





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FIG. 5

BLAST SEARCH RESULTS

	Name	Genbank number	Probability P(N)
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2	Life Tech mouse embryo cDNA 10 5dpc clone 557151	AA111519 (mo51h08.r1)	1.6 e-146
3	Soares mouse 3NbMS cDNA clone 622827	AA184284 (m133b02.r1)	2.0 e-126
4	Stratagene mouse embryonic carcinomaRA cDNA clone 635882	AA105466 (mm92c06.r1)	1.0 e-120
5	WATMI Homosapiens cDNA clone 501115	N2288 (EST501115)	1.4 e-90

FIG. 6(A)

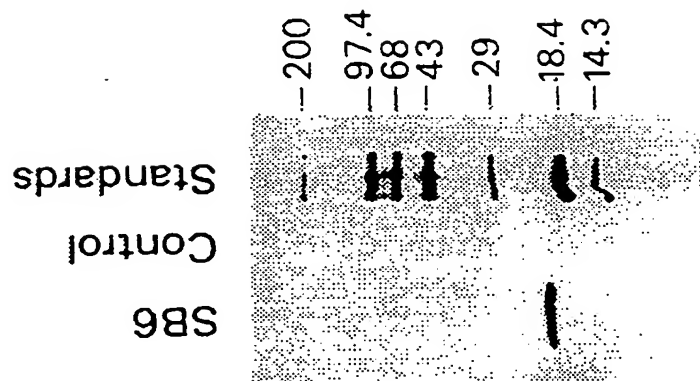
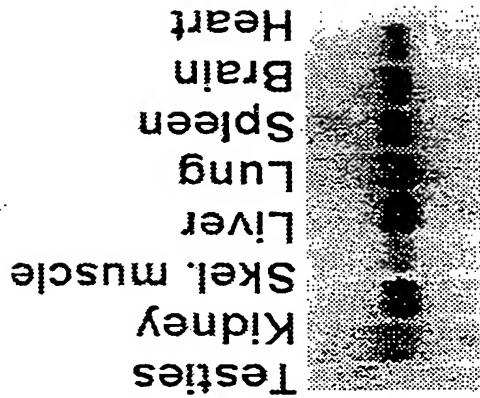


FIG. 6(B)



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FIG. 7

Genomic Southern Blot

Human
Rat
Mouse
Dog
Cow
Rabbit
Chicken
Yeast

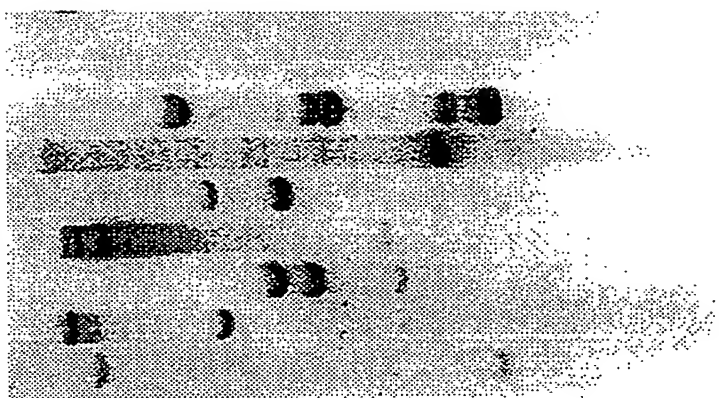


FIG. 8(A)

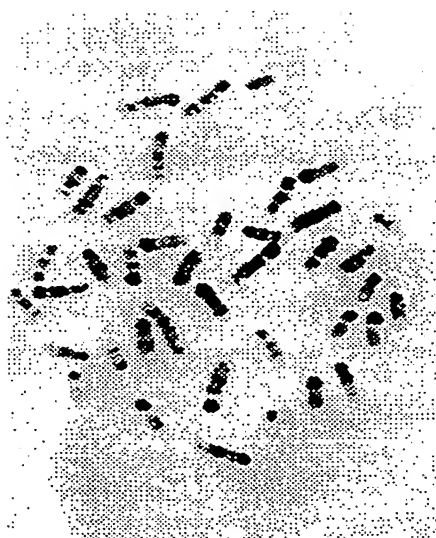


FIG. 8(B)

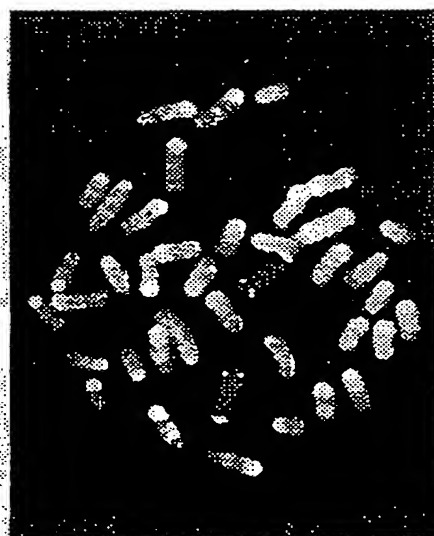


FIG. 8(C)

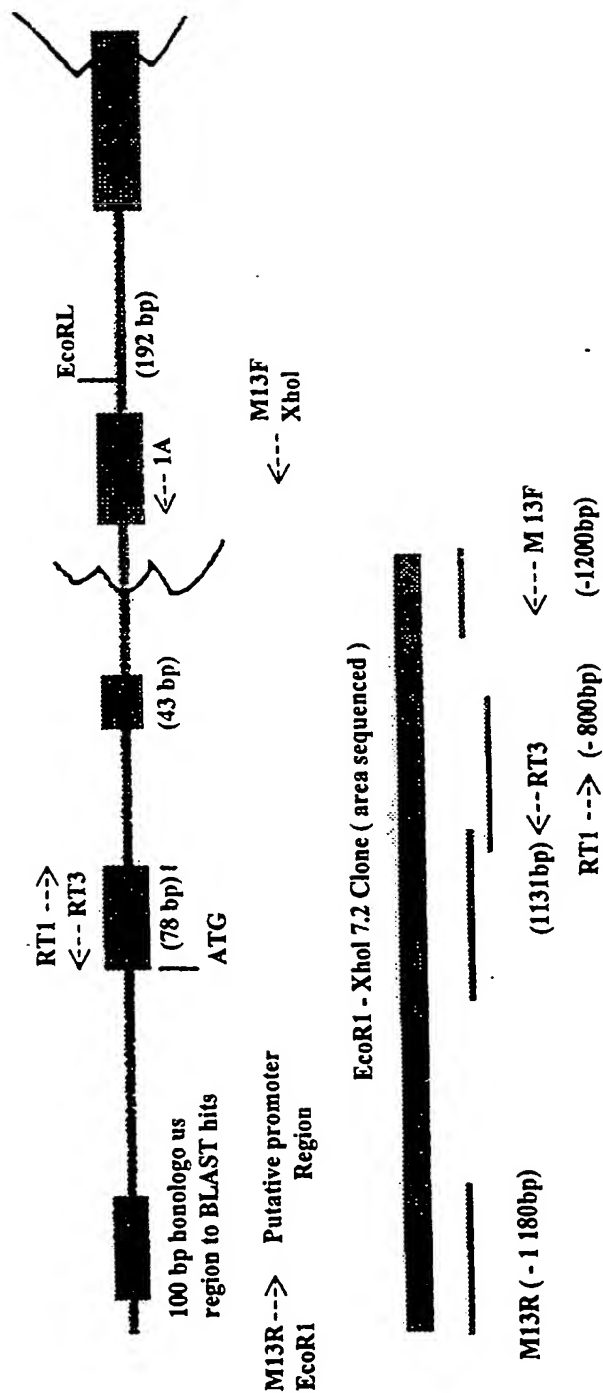


FIG. 9

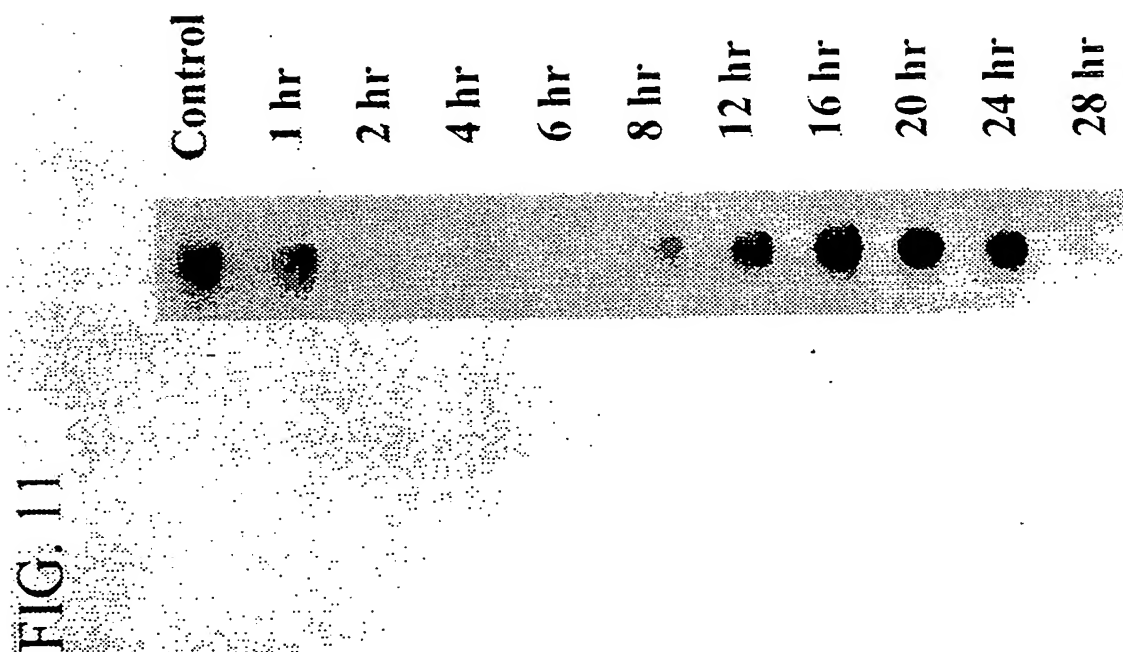


FIG. 9(A)



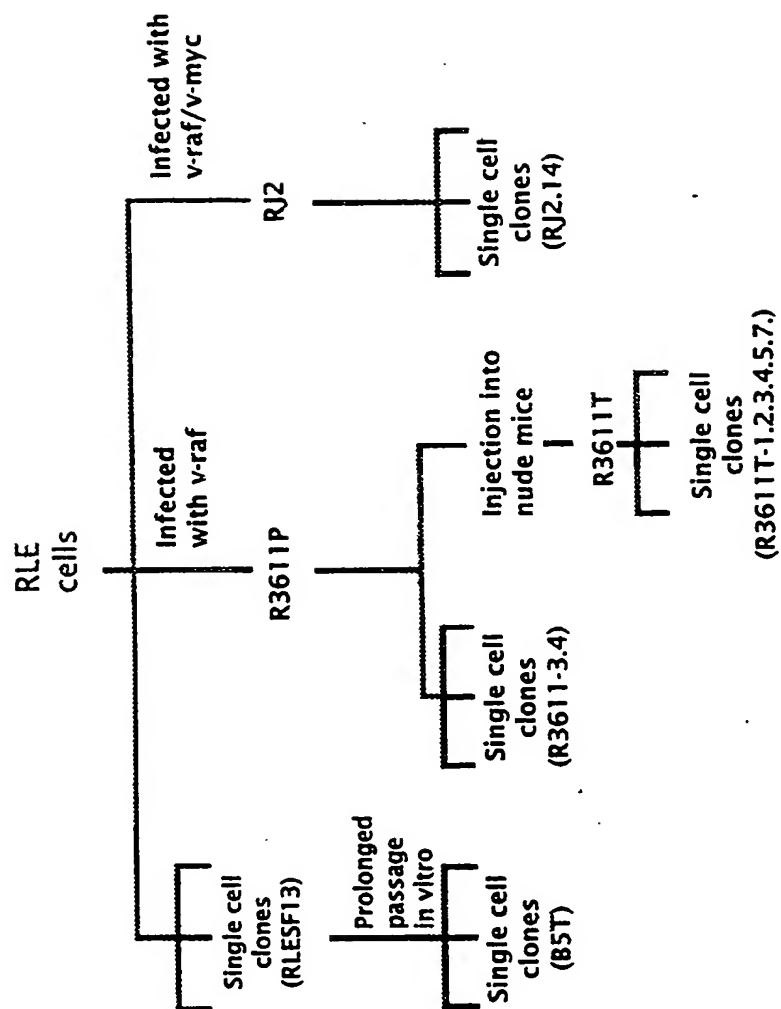
FIG. 9(B)

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FIG. 12



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FIG. 13B

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-----> CPIB (14.00 2.0000)
<----- ACF (14.00 2.0000)

00151 AACCAATGAGCTCCAAATTCACAGATCTTGTGCGAAAACCCAAATGTAAT
-----> C/EBP (10.00 2.0000)
-----> CBF (1) (10.00 2.0000)
-----> CBF (2) (10.00 2.0000)
-----> CBF-A (10.00 2.0000)
-----> CBF-B (10.00 2.0000)
-----> CCAAT-binding factor (10.00 2.0000)
-----> CDF (10.00 2.0000)
-----> CRF (10.00 2.0000)
-----> CTF (10.00 2.0000)
-----> NF-Y (10.00 2.0000)
-----> NF-Y' (10.00 2.0000)
<----- alpha-CBF (10.00 2.0000)
<----- alpha-CP1 (10.00 2.0000)
<----- alpha-CP2a, alpha-CP2b (10.00 2.0000)
<----- alpha-IRP (10.00 2.0000)
<----- CDP (10.00 2.0000)
<----- CDP2 (10.00 2.0000)
<----- Clox (10.00 2.0000)
<----- CP1 (10.00 2.0000)
<----- CP1 (10.00 2.0000)
<----- CP2 (10.00 2.0000)
<----- Cux (10.00 2.0000)
<----- En (16.00 1.6000)
<----- H1TF2 (10.00 2.0000)
<----- NF-1 (10.00 2.0000)
<----- NF-E (10.00 2.0000)
<----- NF-E (10.00 2.0000)
<----- SRF (10.00 2.0000)
<----- TGGCA-binding protein (10.00 2.0000)
-----> HiNF-A (12.00 1.0000)
<----- CP2 (10.00 0.9091)
-----> IUF-1 (10.00 1.6667)
-----> HiNF-A (12.00 1.0000)
<----- AGP/EBP (10.00 1.1111)
<----- AP 3 (2) (13.00 1.6250)
<----- IqPE-1 (16.00 2.0000)
<----- LAP (10.00 1.1111)
<----- NF-IL6 (10.00 1.1111)
<----- NF-IL6beta (10.00 1.1111)

00201 GTGGAATGAAGGAAAAGAAGACCCCAACACTGACTGAATATGGTGACA
<----- HSF (14.00 0.9333)
<----- HSF (14.00 0.9333)
<----- HSF (14.00 0.9333)
<----- HSF1 (long) (14.00 0.9333)
<----- HSF1 (short) (14.00 0.9333)

```

FIG. 13C

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```

<----- HSF1 (14.00 0.9333)
-----> c-Ets-2 (12.00 2.0000)
<----- c-Ets-1 54 (11.00 1.8333)
<----- c-Ets-1 68 (11.00 1.8333)
<----- c-Ets-2 58-64 (11.00 1.8333)
<----- PEA3 (11.00 1.8333)
<----- PEA3 (11.00 1.8333)
<----- PEA3 (11.00 1.8333)
-----> GR (12.00 2.0000)
<----- CACCC-binding factor (10.00 2.0000)
<----- gammaCAC1 (10.00 2.0000)
<----- gammaCAC2 (10.00 2.0000)
-----> AP-1 (16.00 2.0000)
-----> GCN4 (12.00 2.0000)
-----> delta factor (12.00 1.0909)
-----> YY1 (12.00 1.0909)
<----- myc-CF1 (10.00 1.6667)
-----> p300 (14.00 2.0000)
00251 CTCCCTTTTAATGCCAGCACTCAGGAGACAAAAGCAGGCAGATCTTTTG
-----> NF-1/L (10.00 2.0000)
-----> GCN4 (12.00 2.0000)
<----- Zeste (12.00 2.0000)
<----- Zeste (12.00 2.0000)
-----> ADR1 (10.00 2.0000)
-----> LVc (10.00 2.0000)
-----> Zeste (10.00 2.0000)
-----> Zeste (10.00 2.0000)
00301 TGAGTTCTAGGCCAGTCTGGTTTACATAGACAGCTCCAGGCCAGTAAGGG
<----- GR (12.00 2.0000)
-----> NF-IL-2A (16.00 1.6000)
-----> Oct-1 (16.00 1.6000)
-----> Oct-2.1 (16.00 1.6000)
<----- VBP (20.00 1.6667)
-----> NF-E (10.00 2.0000)
<----- T-Ag (10.00 2.0000)
-----> E4BP4 (12.00 1.2000)
00351 GCTAGCTAATGAAACTGTCTTAAACAAATTAACCAACGTTTCATTGAAAA
-----> IUF-1 (10.00 1.6667)
<----- STE12 (16.00 2.0000)
<----- NF-E (10.00 2.0000)
<----- Ftz (18.00 1.6364)
-----> HOXA5 (10.00 1.0000)
-----> c-Ets-2 (12.00 2.0000)
<----- EcR (11.00 1.0000)
-----> TEIID (12.00 2.0000)
-----> Hb (20.00 2.0000)
00401 AAAATAAACCTTCCTTAAAGAAGTATTGGTACAATAAAAAAGATAAC
-----> AGP/EBP (10.00 1.1111)

```


FIG. 13A

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Sequence

RLHEXRT-3

```

00001  CAGAGCCCTGAAAGGTTGTTGCATGAGCCCGTGAAAGTGGAGTTTCAGTG
          <----- NF-W1 (16.00 2.0000)
          <----- NF-W2 (16.00 2.0000)
          <---- Zeste (12.00 2.0000)
          <---- Zeste (12.00 2.0000)
          -----> BAF1 (10.00 0.8333)

00051  GTAGTGGATAGCATAGGACACTGGAGACACAGTTCATGTCCAGCATTCAT
          <----- C/EBP (13.00 1.6250)
          <----- C/EBP (13.00 1.6250)
          <----- C/EBP (13.00 1.6250)
          <----- C/EBP (13.00 1.6250)
          ----> NF-E (10.00 2.0000)
          -----> GR (12.00 2.0000)
          -----> ADRI (10.00 2.0000)
          <----- GR (12.00 2.0000)
          -----> CBF (2) (16.00 1.6000)
          -----> SRF (16.00 1.6000)
          <----- Pit-la (14.00 2.0000)

00101  GGAGTGGGAGCAGAGAGTTCCTGAAGCTCACTGGCTAGTATTCTTGCTA
          <---- GR (10.00 2.0000)
          -----> AGP/EBP (10.00 1.1111)
          -----> C/EBP (11.00 1.2222)
          -----> C/EBP (11.00 1.2222)
          -----> C/EBP (11.00 1.2222)
          -----> C/EBP (11.00 1.2222)
          -----> C/EBP (11.00 1.2222)
          -----> LAP (10.00 1.1111)
          -----> NF-IL6 (10.00 1.1111)
          -----> NF-IL6beta (10.00 1.1111)
          <----- CP2 (10.00 0.9091)
          -----> CPIA (14.00 2.0000)

```

FIG. 13D

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```

-----> c-Ets-1 54      (11.00 1.8333)
-----> c-Ets-1 68      (11.00 1.8333)
-----> c-Ets-2 58-64    (11.00 1.8333)
-----> LAP              (10.00 1.1111)
-----> NF-IL6           (10.00 1.1111)
-----> NF-IL6beta       (10.00 1.1111)
-----> PEA3            (11.00 1.8333)
-----> PEA3            (11.00 1.8333)
-----> PEA3            (11.00 1.8333)
<----- kappaY factor   (16.00 2.0000)
<----- c-Ets-2        (12.00 2.0000)
      <----> alpha-CBF    (10.00 2.0000)
      <----> alpha-CP1   (10.00 2.0000)
      <----> alpha-CP2A, alpha-CP2b (10.00 2.0000)
      <----> alpha-IRP   (10.00 2.0000)
      <----> CDP         (10.00 2.0000)
      <----> CDP2        (10.00 2.0000)
      <----> Clox        (10.00 2.0000)
      <----> CP1         (10.00 2.0000)
      <----> CP1         (10.00 2.0000)
      <----> CP2         (10.00 2.0000)
      <----> Cux         (10.00 2.0000)
      <----> H1TF2       (10.00 2.0000)
      <----> NF-1        (10.00 2.0000)
      <----> NF-E        (10.00 2.0000)
      <----> NF-E        (10.00 2.0000)
      <----> SRF         (10.00 2.0000)
      <----> TGGCA-binding protein (10.00 2.0000)
<----- C/EBP          (10.00 2.0000)
<----- CBF (1)         (10.00 2.0000)
<----- CBF (2)         (10.00 2.0000)
<----- CBF-A          (10.00 2.0000)
<----- CBF-B          (10.00 2.0000)
<----- CCAAT-binding factor (10.00 2.0000)
<----- CDF           (10.00 2.0000)
<----- CRF           (10.00 2.0000)
<----- CTF           (10.00 2.0000)
<----- NF-Y          (10.00 2.0000)
<----- NF-Y'         (10.00 2.0000)
      <-----> HOXA5      (10.00 1.0000)
      <-----> Hb        (16.00 1.6000)
      <-----> GATA-1     (12.00 2.0000)
      <-----> GATA-1     (10.00 1.6667)
      <-----> GATA-1     (10.00 1.6667)
      <-----> GATA-1     (10.00 1.6667)
      <-----> GATA-2     (10.00 1.6667)
      <-----> GATA-2     (10.00 1.6667)
      <-----> GATA-2     (10.00 1.6667)

```

FIG. 13E

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```

                                <----- GATA-2      (12.00 2.0000)
                                <----- GATA-3      (10.00 1.6667)
                                <----- GATA-3      (10.00 1.6667)
                                <----- GATA-3      (10.00 1.6667)
                                <----- GATA-3      (10.00 1.6667)
                                <----- GATA-3      (10.00 1.6667)
                                <----- NF-E1b      (10.00 1.6667)
                                <----- NF-E1c      (10.00 1.6667)
                                <----> DBF-A        (10.00 2.0000)
                                <----- GAL4        (10.00 2.0000)
                                <----- TBP         (10.00 2.0000)
00451  ACATTATGAGCACGCTGTTGCCAGCACATAAGGGATGTGGAGTATGAGAA
                                <----> NF-1/L      (10.00 2.0000)
                                                <----> GCR1      (10.00 2.0000)
                                                <-----> C/EBP      (14.00 1.7500)
                                                <-----> C/EBP      (14.00 1.7500)
                                                <-----> C/EBP      (14.00 1.7500)
                                                <-----> C/EBP      (14.00 1.7500)
                                                <-----> C/EBP      (14.00 1.7500)
00501  GCGTGGAAGAGGGTAAATCAAAGATAATTAATTTGATGGTAATTCAC
<----- AGP/EBP      (13.00 1.8571)
<-----> HNF-A      (12.00 1.0000)
<-----> Kr         (16.00 1.6000)
<----> Pit-1a      (10.00 2.0000)
                                <----> GATA-1      (12.00 2.0000)
                                <----- GATA-1      (10.00 1.6667)
                                <----- GATA-1      (10.00 1.6667)
                                <----- GATA-1      (10.00 1.6667)
                                <----- GATA-2      (10.00 1.6667)
                                <----- GATA-2      (10.00 1.6667)
                                <----- GATA-2      (10.00 1.6667)
                                <----- GATA-2      (12.00 2.0000)
                                <----- GATA-3      (10.00 1.6667)
                                <----- GATA-3      (10.00 1.6667)
                                <----- GATA-3      (10.00 1.6667)
                                <----- GATA-3      (10.00 1.6667)
                                <----- GATA-3      (10.00 1.6667)
                                <----- NF-E1b      (10.00 1.6667)
                                <----- NF-E1c      (10.00 1.6667)
                                <-----> DBF-A        (10.00 2.0000)
                                <-----> HOXA5        (10.00 1.0000)
                                <-----> GAL4        (10.00 2.0000)
                                <-----> TBP         (10.00 2.0000)
                                <-----> N-Oct-3      (12.00 1.7143)
                                <-----> N-Oct-3      (12.00 1.7143)
                                <-----> N-Oct-3      (12.00 1.7143)
00551  AGGTTTGAGTTTAGCTGCCTGTGCTTTAGCCAGAAATGCGTAGGCCTGC
                                <----> Zeste      (10.00 2.0000)

```

FIG. 13F

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-----> Zeste (10.00 2.0000)
               <----- CP2 (11.00 1.0000)
                               -----> Ker1 (16.00 1.6000)
                               <----- LVc (10.00 2.0000)
00601 AGGTATCCAAGAACTACAATTCCCAGAAAGTCCGCAGTGCAGGCTCTGGGC
               -----> GR (12.00 2.0000)
                               -----> AGP/EBP (13.00 1.8571)
                               -----> IL-6 RE-BP (18.00 2.0000)
                               -----> IL-6 RE-BP (18.00 2.0000)
                               <----- Spl (16.00 1.6000)
                               -----> Spl (10.00 2.0000)
                               -----> LVc (10.00 2.0000)
                               -----> GAL4 (10.00 2.0000)
                               -----> GR (10.00 2.0000)
                               <----- T-Ag (10.00 2.0000)
                               -----> GCF (10.00 1.4286)
                               <----- Elk-1 (10.00 1.0000)
                               <----- c-Ets-1 (16.00 1.6000)
                               <----- c-Ets-1 54 (12.00 1.5000)
                               <----- c-Ets-1 68 (12.00 1.5000)
                               <----- c-Ets-1 (12.00 1.5000)
                               <----- PEA3 (12.00 1.5000)
                               <----- PEA3 (12.00 1.5000)
                               <----- PEA3 (12.00 1.5000)
                               -----> Bcd (16.00 1.6000)
                               <----- E1A-F (14.00 2.0000)
00651 CGGATGTAGTCTTGGTCTGAGAGCTGCTGGTCCAAGCTGGGCAAGGTCTC
               -----> GCR1 (10.00 2.0000)
               <----- EFII (18.00 1.6364)
                               <----- H4TF-2 (10.00 2.0000)
                               <----- GCF (10.00 1.0000)
                               <----- T-Ag (10.00 2.0000)
                               <----- LF-A1 (10.00 2.0000)
                               <----- ELP (14.00 2.0000)
                               <----- ADR1 (10.00 2.0000)
00701 CCACGTCTACATTC

```